Comparison of Sex Pheromone Traps for Monitoring Pink Hibiscus Mealybug (Hemiptera: Pseudococcidae)

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ABSTRACT The pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae), is a highly polyphagous pest that invaded Florida in 2002 and has recently been reported from several locations in Louisiana. Although identification of its sex pheromone in 2004 improved monitoring capabilities tremendously, the effectiveness and efficiency of different pheromone trap designs for capturing males has not been evaluated. We deployed green Delta, Pherocon IIB, Pherocon V, Jackson, and Storgard Thinline traps in Homestead, FL, and compared the number of male *M. hirsutus* captured per trap, the number captured per unit of trapping surface area, the amount of extraneous material captured, and the time taken to count trapped mealybugs. Pheromone-baited traps with larger trapping surfaces (green Delta, Pherocon IIB, and Pherocon V) captured more males per trap than those with smaller surfaces (Jackson and Storgard Thinline), and fewest males were captured by Storgard Thinline traps. However, Jackson traps captured as many or more males per square centimeter of trapping surface as those with larger surfaces, and the time required to count males in Jackson traps was significantly less than in green Delta, Pherocon IIB, and Pherocon V traps. Although all trap designs accumulated some debris and nontarget insects, it was rated as light to moderate for all designs. Based on our measures of effectiveness and efficiency, the Jackson trap is most suitable for monitoring *M. hirsutus* populations. Additionally, unlike the other traps evaluated, which must be replaced entirely or inspected in the field and then redeployed, only the sticky liners of Jackson traps require replacement, enhancing the efficiency of trap servicing.

KEY WORDS *Maconellicoccus hirsutus*, sex pheromone, monitoring, trapping

The pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae), has been recorded from many of the world's tropical and semitropical regions (Mani 1989) and is considered a potentially serious pest in the United States, due to its extremely broad range of economically important hosts, including citrus, ornamentals, and vegetables (Hall 1921, Ghose 1972, Stibick 1997) and many potential native hosts (Osborne 2000, Hodges and Hodges 2006). The invasion of numerous Caribbean islands by *M. hirsutus* during the 1990s resulted in widespread damage and significant economic losses (Sagarra and Peterkin 1999). Since its initial detection on hibiscus plants in Broward County, FL, in 2002, pink hibiscus mealybug has spread from Key West to central Florida (Osborne 2000). Between January and July 2004, hibiscus plants from an infested nursery in Homestead, FL, were shipped to 36 states, including some considered climatically suitable for the establishment of the pest, based on the conclusions of several unpublished pest risk assessments (Chang and Miller 1996, USDA 1998, Moffitt 1999, Meyerdirk et al. 2003, Spears et al. 2005). Recent confirmation of pink hibiscus mealybug in several cities in Jefferson Parish, LA (Anonymous 2006) validated the predictions of the aforementioned risk assessments, in which its spread and establishment in the southern United States was anticipated.

In most areas, pink hibiscus mealybug completes a generation in ≈30 d and 10 generations per year are possible (for review, see Mani 1989). In Florida, sex pheromone-based trapping surveys have revealed that males can be captured year-round, but they are most abundant from June through November, with a peak period of activity during midsummer (Hall and LaPointe 2005, Zhang and Amalin 2005).

In response to its arrival in Florida, USDA–APHIS personnel immediately implemented biological control and monitoring programs, and regulatory actions were initiated by the Florida Department of Plant Industry. The ability to effectively and efficiently monitor pink hibiscus mealybug has been enhanced

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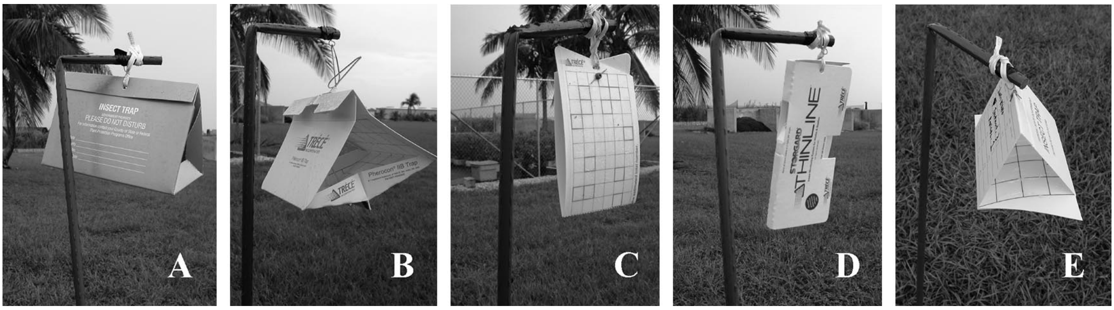

Fig. 1. Traps compared for capturing male pink hibiscus mealybug at Homestead, FL, 2005: (A) green Delta trap, (B) Pherocon IIB, (C) Pherocon V, (D) Storgard Thinline, and (E) Jackson trap.

tremendously by the recent identification, synthesis, and field testing of its sex pheromone (Zhang et al. 2004, Zhang and Nie 2005, Zhang and Amalin 2005).

Given that biological control agents for *M. hirsutus* are very effective but do not eradicate it (unpublished executive summary by Meyerdirk et al. 2003, Roltsch et al. 2006), that regulatory actions dictate zero tolerance for this pest in Florida nurseries, and that it has now been detected in Louisiana, it is likely that ongoing, pheromone-based monitoring of pink hibiscus mealybug will be necessary. To date, green cardboard Delta sticky traps (Fig. 1A) have been used by USDA–APHIS for this purpose. These traps possess a triangular opening on each end and are coated with sticky material on the interior bottom panel and one side, providing a relatively large trapping surface. In 2005, we conducted preliminary monitoring studies in Homestead, FL, by using pheromone-baited green Delta traps and found that pink hibiscus mealybug was captured at all seven sites where the traps were deployed, with considerable variation among sites in the total number captured (Table 1). Although effective for capturing male *M. hirsutus*, we considered the possibility that large Delta traps may not be optimal for this purpose. Males are ≤1.5 mm long (Hall 1921), affecting the ease with which they are identified and counted on traps, particularly those with a large trapping surface. Furthermore, debris and other insects that collect on the sticky trapping surface can cover and hide mealybugs. Finally, if mealybug captures in these Delta traps are recorded at regular sample intervals, each trap must be replaced at the end of each collection period, or each must be opened, inspected in the field, and then redeployed.

Table 1. Total number of male pink hibiscus mealybugs captured in sex pheromone-baited traps deployed in Homestead, FL, 2005

Site	No. traps	No. captured
Univ. of Florida Tropical Research and Education Center[a]	5	642
Golf course 1	1	397
Golf course 2	1	1,601
Municipal park 1	1	174
Municipal park 2	1	2,712
Private residence 1	1	2,244
Private residence 2	1	1,628

[a] Green Delta traps were deployed at the TREC from 29 June to 21 July and at all other locations from 21 July to 11 August.

Two reports have directly or indirectly compared the effect of sex pheromone trap design on the capture of male mealybugs. Although large plate traps captured more citrus mealybug, *Planococcus citri* (Risso), in Israel, Zada et al. (2004) concluded that Delta traps were most suitable for monitoring purposes. A study in California found that Pherocon IIID traps (Trécé, Salinas, CA) captured more male vine mealybug, *Planococcus ficus* Signoret, than the white, two-sided sticky cards traps used to monitor California red scale, *Aonidiella aursanti* (Maskell), and that they also were superior in excluding the hymenopteran parasitoid of the vine mealybug, *Anagyrus pseudococci* Howard (Millar et al. 2002).

Given that the use of sex pheromone-baited traps for monitoring the presence and spread of pink hibiscus mealybug in the southern United States will continue for an indefinite period, the development of optimally efficient and standardized trapping protocols is warranted. Toward that end, we compared the effectiveness and efficiency of five commercially available sex pheromone traps, based on the capture of male *M. hirsutus* and on other criteria associated with the logistics of their use.

Materials and Methods

Traps and Trapping Locations. The five traps evaluated were 1) large cardboard Delta (Scentry Biologicals, Billings, MT), 2) Pherocon IIB (Trécé), 3) Pherocon V (Trécé), 4) Storgard Thinline (Trécé), and 5) Jackson (Trécé) (Fig. 1A–E). As well as representing a range of sizes, trapping surface areas and designs, these traps were selected based on potential differences among them in ease of servicing and processing and in the number of nontarget insects and debris that they would accumulate. Gray, halo-butyl septa (5 mm; West Pharmaceutical Services, Kearney, NE) loaded with 1 μg of the sex pheromone of pink hibiscus mealybug (Zhang et al. 2004) were secured to Pherocon V and Storgard Thinline traps at the lure insertion hole manufactured into each. Lures were suspended within the center of the interior of Pherocon IIB, Delta, and Jackson traps with 22-gauge wire and were ≈3 cm above the trap floor. Blank septa were affixed to control traps of each design.

Trapping was conducted at the University of Florida, Tropical Research and Education Center (TREC) in Homestead, FL, and at two residential locations in Homestead from 2 September until 14 October, 2005. This trapping interval was based on the seasonal phenology of the pest; in south Florida, populations peak during the summer and are abundant through November (Hall and Lapointe 2005; Zhang and Amalin 2005). Traps were suspended from the end of a 90° bend in metal stakes (Fig. 1) that were embedded in soil and rocks within three gal plastic containers and were suspended at a height of 1.2 m above the ground. Each of the five trap designs were represented twice (pheromone-baited and blank control) at each location and the 10 traps were randomly assigned to positions within a straight line (3 m between traps) that was perpendicular to the prevailing easterly wind direction in south Florida. At weekly intervals, the green Delta, Pherocon IIB, Pherocon V, and Storgard Thinline traps were replaced, whereas only the sticky liners of Jackson traps were replaced, and the treatments were rotated among positions within each row at each of the three locations.

Evaluation of Traps. The traps and trap liners collected each week were covered with clear plastic wrap and the male pink hibiscus mealybugs captured in each were counted using a dissecting microscope at 30× magnification. The time taken to count all male mealybugs on each trap was recorded using a stop watch.

During a pheromone trap-based study earlier in 2005 (Zhang et al. 2006), subsamples of male mealybugs were removed from all traps, slide mounted, and keyed to species, based on the presence of a sclerotized loop above the genital capsule (Hodges and Hodges 2006), and all were confirmed as *M. hirsutus.* Consequently, for the current study, subsamples of ≤10 males per trap were processed and identified after the first week of sampling and not thereafter.

After the male *M. hirsutus* on each trap had been counted, traps were evaluated for the presence of debris and larger nontarget insects and the total amount of extraneous material covering the sticky surface. The amount of debris (e.g., dirt particles and leaves) was rated using a qualitative scale: 0, none; 1, light; 2, moderate; and 3, heavy. Larger, nontarget insects that could potentially obscure *M. hirsutus* males on the trapping surface were assigned to two categories, Lepidoptera and insects >5 mm long. The total amount of extraneous material was rated according to the same qualitative scale as described above, and included debris, Lepidoptera, insects >5 mm long, and all insects <5 mm long.

Tests for normality (PROC UNIVARIATE, SAS Institute 2001) were conducted on all data sets, revealing that the assumption of homogeneity of variance was met for all data except the number of males per trap, which, based on the shape of the scatterplot, were subjected to a logarithmic transformation (base 100).

Due to variation among trap designs in the trapping surface area, comparisons of the capture of male mealybugs among them used both the total number captured and the number captured per square centimeter of trapping surface. Calculations of the number of males captured per square centimeter of trapping surface used a single value for the total trapping surface area for each trap design, based on measurements from a single trap considered to be representative of each design. Comparison of the mean total number of males per trap (pheromone-baited and blank controls) among trap designs used sites as replicates (n = 3) and was based on one-way analysis of variance (ANOVA) (PROC ANOVA, SAS Institute 2001) of log-transformed data, followed by Fisher protected least significant difference (LSD) test. Comparisons of the number of males per square centimeter of trapping surface and the time to count males used individual, pheromone-baited traps as replicates (n = 18; three sites × 6 wk) and relied on the same tests. Because many traps were given 0 ratings for the amount of debris, number of Lepidoptera and number of insects >5 mm long, these data are presented using only descriptive statistics and are based on pheromone-baited traps only. Comparisons of ratings of the total amount of extraneous material per pheromone-baited trap used individual traps as replicates (n = 18) and were based on one-way ANOVA and Fisher protected LSD tests. All statistical comparisons were considered significantly different at the 5% probability level.

Results and Discussion

Preliminary trapping studies in 2005 confirmed that pink hibiscus mealybug is widely established in the Homestead area (Table 1) and revealed apparent differences in population densities among locations, although infestations were not observed on host vegetation in the vicinity of traps at any location.

All mealybugs subsampled from pheromone-baited traps on 9 September were identified as *M. hirsutus*, concurring with our previous findings and further confirming the species specificity of the pheromone (Zhang and Amalin 2005). There were apparent differences in the size of *M. hirsutus* populations among the three sites at which trap designs were compared; total captures were 57, 1,496, and 1,593 males at sites 1, 2, and 3, respectively. These presumed differences in populations were associated with large differences in trap captures and introduced considerable variability that likely influenced the results of statistical comparisons of total captures among pheromone-baited and blank traps. Comparison of the log-transformed total number of male *M. hirsutus* captured in pheromone-baited and blank traps revealed significant differences among them ($F = 6.29$; df = 9, 82; $P < 0.0001$) (Table 2). Blank traps of all designs caught relatively few mealybugs but showed that some males apparently do enter them passively; concurring with the results of previous studies (Zhang et al. 2004, Zhang

Table 2. Effect of trap design on the capture of male pink hibiscus mealybug in Homestead, FL

Trap design	Mean ± SE no. of pink hibiscus mealybugs captured	
	Sex pheromone lure	Blank lure
Green Delta	353.7 ± 166.4a	3.7 ± 3.2c
Pherocon V	248.3 ± 124.4ab	5.7 ± 2.8c
Pherocon IIB	214.3 ± 101.9abc	8.0 ± 2.5c
Jackson	156.7 ± 77.4abc	11.3 ± 6.1c
Storgard Thinline	66.7 ± 33.6bc	4.3 ± 3.4c

Means for all pheromone-baited and blank traps followed by the same letter(s) are not significantly different at $\alpha = 0.05$, based on log-transformed data. Means are based on one baited and one blank trap of each design deployed at each of three sites from 2 September to 14 October 2005.

and Amalin 2005). Pheromone-baited green Delta traps caught significantly more males than baited Storgard Thinline traps. The mean total number of males captured did not differ significantly among the green Delta, Pherocon V, Pherocon IIB, or the Jackson trap (Table 2).

Based on qualitative comparisons of the percentage of total male capture among sites by traps of each design, green Delta, Pherocon IIB, and Pherocon V traps seemed to perform similarly among sites and independently of presumed differences in the size of populations among sites (Fig. 2). Jackson traps and Storgard Thinline traps seemed to perform similarly at sites 2 and 3, but they showed differences in the percentage of total male capture at site 1, where total captures were lowest (Fig. 2). The difference in the actual number of males captured between these two trap designs at site 1 was not pronounced (three and 11 for Jackson and Storgard Thinline, respectively), and the small total sample size at that site ($n = 57$ males) likely skewed the percentages of total capture, relative to the other sites. Based on our continued use of Jackson traps in 2006, we do not think that the differences among sites recorded in 2005 reflect a difference in the ability of Jackson traps to detect different population densities of pink hibiscus mealybug. In a survey intended to identify study sites with differing mealybug populations, a single, pheromone-baited Jackson trap was deployed at 50 locations around Homestead, FL, from 25 May through 8 June 2006, including residential sites with hibiscus and open sites not in proximity to host plants. No males were captured at the three open sites without host plants, whereas captures were recorded from all other sites, ranging from 1 to 1,320 males per site (mean = 207) (J.V., unpublished data).

Table 3. Trapping surface area, mean no. of male pink hibiscus mealybugs (PHM) captured per unit of trapping surface area and the mean time to count male pink hibiscus mealybugs in five trap designs

Trap design	Trapping surface area (cm^2)[a]	Mean ± SE no. PHM trapped/cm^2	Mean ± SE no. seconds/trap to count PHM
Green Delta	340.0	0.17 ± 0.04ab	158.4 ± 6.8a
Pherocon V	187.5	0.22 ± 0.05a	137.2 ± 8.7b
Pherocon IIB	476.0	0.08 ± 0.02b	175.3 ± 5.5a
Jackson	115.0	0.20 ± 0.05a	73.4 ± 4.2c
Storgard Thinline	150.0	0.07 ± 0.02b	72.7 ± 4.6c

Means within columns followed by the same letter(s) are not significantly different at $\alpha = 0.05$. Means are based on weekly data from each pheromone-baited trap, collected over 6 wk at three sites ($n = 18$ per trap design).

[a] The sticky trapping surface area was calculated based on measurements from a single, representative trap of each design, and this measurement was used in all calculations of the number of PHM males trapped per square centimeter for each trap design.

The trapping surface area of the five trap designs differed considerably (Table 3), with about a 3 times difference between the smallest (Jackson) and the largest (Pherocon IIB) traps. Comparisons of the mean number of males per square centimeter of trap-

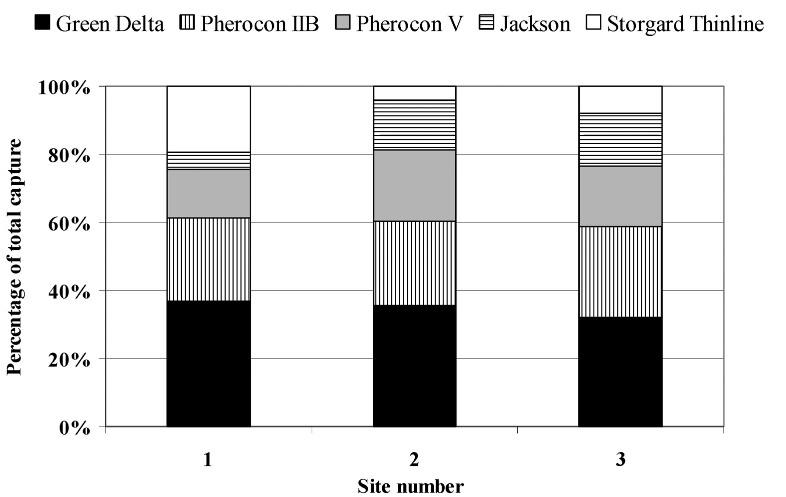

Fig. 2. Percentage of total capture of male pink hibiscus mealybug among five trap designs deployed at three sites in Homestead, FL, from 2 September until 14 October, 2005. Total number of males captured were 57, 1,496, and 1,593 males at sites 1, 2, and 3, respectively.

Table 4. Effect of trap design on the capture of extraneous objects and nontarget insects in traps used to capture male pink hibiscus mealybug

Trap design	Mean ± SE debris rating/trap[a]	Mean ± SE no. insects >5 mm/trap	Mean ± SE no. Lepidoptera/trap	Mean ± SE rating of total extraneous objects/trap[b]
Green Delta	0.11 ± 0.08	1.28 ± 0.32	0.56 ± 0.26	0.61 ± 0.14a
Pherocon V	0.33 ± 0.14	0.33 ± 0.14	0.0	1.72 ± 0.19c
Pherocon IIB	0.17 ± 0.09	2.61 ± 0.59	0.83 ± 0.27	1.22 ± 0.17b
Jackson	0.33 ± 0.11	2.0 ± 0.33	0.17 ± 0.12	1.44 ± 0.17bc
Storgard Thinline	0.06 ± 0.06	0.39 ± 0.16	0.0	0.67 ± 0.14a

Means for all ratings and counts are based on data from each pheromone-baited trap from each week of trapping over 6 wk at three sites (n = 18 per trap design).

[a] Debris included nonliving objects such as dust, sticks and leaves and was rated according to a qualitative scale from 0 (none) to 3 (heavy).

[b] Total extraneous objects included the combined effects of debris, insects >5 mm long, Lepidoptera, and insects <5 mm long and was rated on the same scale as described above. Means followed by the same letter(s) are not significantly different at $\alpha = 0.05$.

ping surface indicated significant differences in their relative efficiency ($F = 3.21$; df = 4, 85; $P < 0.02$). Jackson and Pherocon V traps captured significantly more males per unit of trapping surface than Pherocon IIB or Storgard Thinline traps, whereas there were not differences among the Jackson, green Delta, or Pherocon V traps (Table 3). Differences in the trapping surface area among trap designs also resulted in significant differences among them in the time taken to locate and count the male mealybugs captured ($F = 59.73$; df = 4, 85; $P < 0.001$). Counting mealybugs on green Delta and Pherocon IIB traps took significantly longer than on the other traps (Table 3). Counting time on Pherocon V traps was significantly longer than on Jackson and Storgard Thinline traps, with no difference in the counting time between the latter two designs.

Some traps of all designs accumulated debris, although debris was minimal on Storgard Thinline traps (Table 4). Fewer insects >5 mm were captured in the Storgard Thinline and Pherocon V traps, compared with the other traps, and no Lepidoptera were recorded from Storgard Thinline or Pherocon V traps, whereas all other designs captured moths and butterflies. Ratings of total extraneous objects showed significant differences among trap designs ($F = 8.69$, df = 4, 85; $P < 0.0001$). Green Delta and Storgard Thinline traps accumulated significantly less extraneous material than the other traps (Table 4), although the average rating for the other trap designs indicated only light to moderate accumulation of other objects.

Compared with many insect pests, monitoring mealybugs is particularly challenging due to their small size and to a general lack of data for developing sampling programs (Geiger and Daane 2001). Given that pink hibiscus mealybug is now established in Florida and Louisiana and that its continued spread represents a potential threat to other southern states, the further development of pheromone traps as a quantitative and predictive sampling tool for pink hibiscus mealybug is warranted and should be based on a standardized protocol using the most efficient and effective trap. The combined results of our comparison of several common trap designs leads us to conclude that the Jackson trap may be best suited for this purpose. An additional compelling reason for this conclusion is the removable and relatively small sticky liner used with the Jackson trap. From a logistical perspective, this is a superior feature that should maximize the ease of trap servicing, compared with traps that must be replaced or inspected in the field and then redeployed at each sample interval. Furthermore, pheromone lures deployed in Jackson traps do not need to be transferred to new traps, as is the case when traps of other designs are replaced at each sample interval. The smaller trapping surface area presented by the Jackson trap does not adversely affect captures, as indicated by the equal or higher numbers of males per unit of trapping surface compared with other traps presenting a larger sticky surface. The smaller trapping surface of Jackson trap liners is more quickly scanned than most of the other traps that we evaluated, and the liners are more easily manipulated on the stage of a dissecting microscope than are larger traps. Finally, there was not a meaningful difference between the Jackson trap liner and other traps in the amount of extraneous material accumulated that could impede the location and counting of male pink hibiscus mealybugs.

Recent studies by Millar et al. (2002) report significant correlations between the capture of male citrus mealybugs in sex pheromone traps, visual estimates of population density, and economic damage. The correlation of captures in pheromone traps with infestation levels of pink hibiscus mealybug has yet to be established but should be given priority. Furthermore, the correlation between population density and the number of male *M. hirsutus* captured in traps may relate to the number of aerially dispersing mealybug crawlers. Investigation of this relationship may yield valuable information toward assessing and predicting the risk of infestation of commercial ornamental nurseries via mealybug dispersal.

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